Module 4a: Microbiological Testing—E. coli

Host

As you know from our earlier discussion, one part of the new pathogen reduction rule requires slaughter establishments to conduct routine *E. coli* testing of carcasses. Let's find out exactly what industry's role is regarding this new requirement.

Establishments are required to maintain sanitary conditions and use good manufacturing practices to avoid contamination with visible feces and ingesta and the bacteria associated with these materials. Fecal contamination is one of the principal sources of pathogenic organisms that contaminate carcasses. The single best indicator of fecal contamination is *Escherichia coli* because it's commonly found in the intestinal tract of food animals.

Under the new rule, establishments are required to have a written program before conducting testing for generic *E. coli,* more specifically known as *E. coli*, Biotype I. The program must address five topics: the procedures used for collecting samples, which employees will collect the samples, the locations in which samples will be collected, how randomness is achieved in sample selection, and how samples will be handled to ensure sample integrity.

Plant employees must conduct *E. coli* testing on meat and poultry carcasses. FSIS employees will oversee the plant's testing program but will not conduct *E. coli* testing themselves.
E. coli testing is a plant verification activity designed to supplement the FSIS organoleptic inspection for removal of visible contamination. This microbial testing verifies that the slaughter process for removing fecal material and ingesta is controlled.

E. coli testing is not used as a performance standard for industry. It is, instead, an indicator of sanitary dressing process control in slaughter establishments. *E. coli* test results are compared to performance criteria developed by the Agency from results of its Microbiological Baseline Studies. The criteria were designed to help each establishment determine whether its process control methods to reduce carcass contamination with feces and ingesta are effective.

The Agency is still conducting tests to develop more performance criteria for *E. coli* testing. In some cases data collected from industry might even be used to develop these additional performance criteria. As results of additional microbiological surveys become available, criteria will be updated. In those cases where the Agency hasn't yet provided performance criteria, establishments must use statistical process control to evaluate their test results.

E. coli performance criteria are not enforceable regulatory standards. Test results that show an establishment meets or beats the results of Agency performance criteria simply provide evidence that the establishment is maintaining adequate process control for fecal contamination. You **will not take regulatory action** based only on a plant's failure to meet performance criteria.

As of August 1997, not all species must be tested for generic *E. coli*. The 1997 requirements for *E. coli* testing apply only to establishments that slaughter all market classes of cattle, swine, chickens and turkeys. Cattle includes calves, cows, bulls, steers, heifers, and hide-on calves. Swine includes sows, boars, gilts, market hogs, suckling pigs, and pigs. Chickens include broilers, light and heavy fowl, Cornish hens, and capons. Turkeys include hens, toms, heavy and light breeders, and young turkeys.

This means that, as of August 1997, establishments slaughtering sheep, goats, horses, ratites, quail, rabbits, and ducks are not required to test for *E. coli*.

If an establishment slaughters combinations of types of livestock or poultry it will only sample from the species it slaughters in the largest number. For example, if an establishment slaughters both cattle and swine, and it's yearly average shows that it slaughters mostly cattle, then it will conduct *E. coli* testing only on cattle. This is because samples from one type of livestock or poultry will provide sufficient information to verify establishment process controls over sanitary dressing.

In some plants the major species slaughtered might fall under one of the species that doesn't require testing. In that case, that establishment won't be required to conduct *E. coli* testing on that species, but would be required to test the species specified in the regulations. For example, an establishment that slaughters mostly sheep and some cattle will be required to conduct *E. coli* testing on the cattle.

For *E. coli* testing requirements, the Agency divided slaughter establishments into two size categories: very low volume plants and greater than very low volume plants. Plant size is based on the average annual slaughter volume in 1996. Each plant is responsible for deciding into which size category it fits.

FSIS defines a very low volume cattle slaughter plant as one that slaughters less than six thousand cattle annually. Therefore, establishments slaughtering more than six thousand cattle are considered greater than very low volume plants. Similarly, very low volume swine slaughter plants annually kill less than twenty thousand swine. Very low volume combination establishments, slaughtering both cattle and swine, annually slaughter less than a combination of six thousand cattle and twenty thousand animals total. Very low volume chicken slaughter plants slaughter less than four hundred forty thousand chickens annually. Very low volume turkey slaughter establishments annually slaughter less than sixty thousand turkeys. And, finally, very low volume chicken and turkey combination slaughter plants annually slaughter less than a combination of sixty thousand turkeys and four hundred forty thousand birds total.

Implementation dates for *E. coli* testing are based on plant size. Greater than very low volume slaughter establishments began *E. coli* testing first on January 27, 1997. Very low volume establishments started *E. coli* testing during the first full week they operated after June 1, 1997.

The *E. coli* regulation also stipulates testing frequencies. Greater than very low volume establishments must sample one of each three hundred cattle carcasses, one of each one thousand swine carcasses, one of each twenty-two thousand chicken carcasses, one of each three thousand turkey carcasses, or a minimum of once per week for any species, whichever is greater. For example, an establishment that slaughters nine thousand cattle would sample once per week resulting in collecting fifty-two samples per

year, not thirty samples as required by the one test per three hundred carcasses frequency.

Plants slaughtering fifty or fewer animals per year will sample no more than twenty-five percent of its carcasses.

In some cases an establishment operating under a validated HACCP plan may substitute an alternative frequency for the frequency published in the regulation. This is allowed only if the alternative frequency is already part of the establishment's HACCP verification procedures. Establishments not under a HACCP plan will have to test at the frequencies specified in the regulation unless they obtain an exemption from the Office of Policy, Program Development, and Evaluation in Washington, D.C.

You'll see one of three sampling methods used by plant employees collecting *E. coli* samples. The three methods are excising, sponging, and whole bird rinsing.

Excision sampling involves aseptically cutting an eight by six-inch by one-half inch thick surface section from the carcass and sending the excision sample for laboratory analysis. Excising tissue from a carcass is, of course, a destructive method of sampling.

Sponging involves aseptically swabbing a sterile sponge on a ten centimeter by ten centimeter surface of the carcass and sending the sponge to the laboratory for analysis. Sponging is a nondestructive method of sampling.

Whole bird rinsing involves shaking the whole carcass in a bag containing buffer solution, collecting the rinse fluid, and sending it to the laboratory for analysis.

How will you know who is allowed to use which method or methods? Establishments slaughtering cattle and swine may choose either excision sampling or sponging. Establishments slaughtering chickens must use the whole bird rinse. Establishments slaughtering turkeys may use either the whole bird rinse or sponging.

FSIS assumes that meat plants following FSIS guidelines will sample in manner that doesn't jeopardize the integrity of the sample or the reliability of the test results. The "Guidelines for *E. coli* Testing for Process Control Verification in Cattle and Swine Slaughter Establishments" and the "Guidelines for *E. coli* Testing for Process Control Verification in Poultry Slaughter Establishments" are both available to plants. Of course these guidelines are not a regulatory requirement, so if the plant chooses to use a comparable sampling technique they're not out of compliance. Copies of the sampling guidelines for both cattle and swine and for poultry are in your workbook at the end of Module 4a.

To familiarize industry with *E. coli* sampling guidelines, the Agency also produced two videos for distribution to individual establishments by their trade groups. They discuss sterile technique and illustrate the sponge sampling technique for cattle and swine and for poultry.

(CATTLE SAMPLING VIDEO)

*That's how industry personnel may sample livestock for *E. coli* testing. Let's go next to sampling poultry. Although you'll again see FSIS inspectors taking the samples,

remember that this is an establishment's responsibility. Only plant employees take these samples for *E. coli* testing.

*A whole-bird rinse is used for poultry samples. But, the plant may elect to sponge turkey carcasses, which are often too large to easily rinse as a whole carcass.

*The poultry sampling supplies are slightly different from those used to sample livestock. All supplies must be sterile.

For a whole bird rinse you'll need a pair of gloves, a large bag, four hundred milliliters of sampling solution for chickens, six hundred milliliters of sampling solution for turkeys, a zip-lock bag, and a sealed container. For sponging the turkey carcass, you'll need two pair of gloves, a sponge in a whirl-pak bag, ten milliliters of sampling solution, and a five by ten centimeter template.

Examine the sampling solution carefully a day or so before use. If it's cloudy, turbid, or has particulate matter in it, don't use it – it's contaminated with bacteria. Properly dispose of this solution. If it's clear, keep it refrigerated for at least twenty-four hours up until just before using it.

All sample units have to be randomly selected. This means that each sample unit has an equal chance of being chosen. A sample unit is a whole, intact carcass. This means the carcass must not have any trimmed areas, whether or not the neck is attached. You'll need to include your random method for sample collection in the written procedures.

Before selecting the sample, you need to determine the sampling location. This location must be safe and accessible. It should be in an area at the end of the drip line after chilling and before packing and cut-up. Because hot-boned turkeys are boned before chilling, they should be sampled between the final wash and the chiller. This is in place of sampling at the end of the drip line and pertains only to hot boned carcasses.

*Remember the importance of aseptic sampling. Do not contaminate sterile surfaces of the sampling supplies.

The first step in aseptic sampling is to wash any surface to be sanitized, since sanitizers are only effective when applied to clean surfaces. If possible, sanitize the surface on which you will place your sampling supplies. Next, sanitize your tote or caddy, if you use one.

After you've prepared your supplies, wash and sanitize your hands up to the mid-forearm. Dry them thoroughly with paper towels. Just before you actually collect the sample, you'll need to put on sterile gloves.

*Recall the proper way to aseptically put on gloves that was shown in the livestock video. We'll let you know during the sampling sequence at just what point you put on the gloves.

*Okay, so let's quickly review sampling poultry for *E. coli*. First, gather your sampling supplies, then wash and sanitize your work surfaces and your hands. Go to the location where you will collect the bird. Count back five and take the sixth bird. But only take it if it is intact. Do not choose a trimmed carcass. Whether or not it has a neck isn't important.

Notice here that he did not take the original sixth bird, since it was a trimmed carcass. He continued counting back five until he got one that was **untrimmed**.

Now here's a look at sampling…

(POULTRY SAMPLING VIDEO)

Return the turkey to the point where you collected; then, clean up your supplies and store the sample.

*Recall from the livestock video that the samples must be kept refrigerated, never frozen, until they are either analyzed by the in-plant lab or shipped.

*This concludes the sampling portion of industry's role in *E. coli* testing.

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If more than one shift is operating at the plant, the sample can be taken from either shift provided the sample selection time is based on the appropriate sampling frequency.

After samples are collected they must be analyzed. The establishment may analyze their samples in their own in-house laboratory or they may ship the samples to an outside laboratory. Test results must come from one of the generic *E. coli* quantitation methods found in the Official Methods of Analysis of AOAC INTERNATIONAL or from a method that is approved and published by a scientific body.

Establishments must keep records of the *E. coli* test results. Each test result must be recorded in terms of colony forming units per square centimeter for excision and sponging results and in colony forming units per milliliter when whole bird rinses are tested.

Since *E. coli* performance criteria haven't been developed for all types of sampling, the method of sampling an establishment uses will affect the type of comparison data it uses and how its records look.

Remember that cattle and swine establishments may choose either excision of three sites or nondestructive sponging of three sites when taking samples. If excision sampling is selected the establishment must use FSIS's performance criteria of little m and big M to evaluate test results.

If a cattle or swine establishment selects the sponging method, Agency performance criteria aren't yet available, so it must use statistical process control to evaluate test results.

Chicken slaughter establishments don't have a choice of sampling methods. They must use the whole bird rinse method and are required to use the "m" and "M" performance criteria established by the Agency's microbiological baseline studies.

Turkey slaughter plants may use either the whole bird rinse or sponging method. However, since baseline studies haven't been established for turkeys, all turkey slaughter establishments must use statistical process control to evaluate test result data.

FSIS plans to complete its baseline studies to cover the sponging method in cattle and swine and both turkey sampling methods around the middle of 1998. When these studies are complete, “m” and “M” values will be determined, and performance criteria will be provided to slaughter establishments.

I keep talking about “m” and “M” values. Let’s talk about the Agency performance criteria known as little m and big M. Just what do they represent? Keep in mind that the *E. coli* performance criteria—“m” and “M”—are not enforceable regulatory standards. They’re only guidelines for establishments and FSIS to use in ensuring that fecal material, ingesta, and associated bacterial contamination is prevented or reduced on carcasses.

For process control verification *E. coli* test result levels are separated into three categories: acceptable, marginal, and unacceptable. Little m represents the marginal range of test results. Marginal results are those within the worst twenty percent of overall industry performance in terms of *E. coli* counts. More than three marginal results in the last thirteen tests is unacceptable.

Big M represents the unacceptable limit of test results. *E. coli* counts higher than ninety eight percent of the establishments in the national baseline study are represented by big M. In other words, big M represents the worst two percent of industry performance. Any single *E. coli* test result exceeding big M is unacceptable.

These performance criteria are published in the regulations.

To illustrate the use of these performance criteria, consider a steer/heifer slaughter establishment. *E. coli* test results for this establishment are acceptable if they come back negative, marginal if the test result is positive but not above one hundred colony forming units per square centimeter and unacceptable if they area above one hundred colony forming units per square centimeter..

The company may document its test results in table form, in chart form, or in both forms.

Charts or tables of the sample result data must show at least the most recent thirteen test results. These thirteen tests are called the moving window, because the oldest test is dropped when the latest test results are documented and the window moves forward by one space. The window of thirteen tests continues to move to reflect only the most up-to-date information.

As I mentioned earlier, cattle and swine slaughter establishments that use the sponging technique for sampling and all turkey slaughter establishments won’t use the “m” and “M” performance criteria because the Agency hasn’t completed its baseline studies for these types of sampling. In the meantime, these establishments are still required to document their test results, so FSIS decided they must use an acceptable method of statistical process control to document their results. The company will essentially conduct its own baseline study and set its own acceptable range and upper control limit. If test results recorded on the company chart stay within the control limits set by the company, the process is considered in control.

There are certain elements common to statistical process control charts. The test result is plotted against time. The centerline indicates the center point of the range considered acceptable by the company. The upper control limit line marks the highest test result

value that is considered acceptable by the company. Under the new rule, official establishments are not required to maintain a file of laboratory reports received from either an in-house laboratory or an outside laboratory. They are only required to keep a table or a chart of the results.

Whenever a plant determines that its *E. coli* test results do not meet m and M performance criteria it must take corrective action to bring the process back into control. In the case of plants using statistical process control, when E*. coli* test results do not meet *E. coli* limits set by the plant,

corrective action to regain process control must be taken. Although the plant is required to make corrections to its process to regain control of contamination, it is **not** required to document those corrective actions.

HOST

We've covered several topics related to *E. coli* testing. Recall that industry is responsible for developing a written program for *E. coli* testing. They must also collect and analyze samples, and they must keep records of *E. coli* test results. All of these topics, plus more details about plant *E. coli* testing, can be found in Module 4A of your notebook.

We've looked at the plant's responsibility for *E. coli* testing. The second part of the pathogen reduction rule addresses requirements for *Salmonella* testing in official establishments. For this part of the rule, FSIS inspectors will conduct the testing.